

REMARKS

Claims 1-20 and 58-79 are pending and under examination. Claims 1 and 59 have been amended. Support for the amendments can be found throughout the specification and the claims as filed. In particular, support for the amendment to claims 1 and 59 can be found, for example, on page 16, line 26, to page 17, line 16, and page 45, lines 7-14. The preamble of claims 1 and 59 have also been amended to provide antecedent basis for the claims as amended. Accordingly, these amendments do not raise an issue of new matter and entry thereof is respectfully requested.

Regarding the Priority Claim

Regarding the priority claim, Applicants respectfully disagree with the assertion in the Office Action on page 2 that parent provisional application serial No. 60/209,539, filed June 5, 2000, fails to provide an enabling disclosure for the claimed methods. As discussed below, Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Priority should be granted to the June 5, 2000, filing date of parent application 60/209,539.

Rejection Under 35 U.S.C. § 112, First Paragraph

The rejection of claims 1-20 and 58-79 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods.

Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance for the claimed methods of differentiating cells to produce a cell population containing neuronal cells protected from apoptotic cell death both *in vivo* (claims 1-20 and 58) and *in vitro* (claims 59-79). Applicants maintain the position of record, in particular as discussed in the previous response filed February 23, 2006, regarding the references asserted to support a lack of enablement. Briefly, Applicants maintain that the issues that may exist for generating homogeneous populations of neural stem cells or the functional integration of donor neural stem cells and their biological properties, as discussed in Rossi and Cattaneo, Nat. Rev. Neurosci. 3:401-409 (2002), are not relevant to the claimed methods producing protected neuronal cells. In the Office Action on page 10, it is asserted that Applicants dismiss

the teachings of Rossi and Cattaneo as not relevant to the claimed methods “because Rossi and Cattaneo discuss neural stem cells.” As reiterated above, it is not the discussion of neural stem cells, per se, that are irrelevant, rather, it is issues that may exist for generating homogeneous populations of neural stem cells or the functional integration of donor neural stem cells and their biological properties as discussed in Rossi and Caettaneo that are not relevant to the claimed methods of differentiating cells to produce a cell population containing neuronal cells protected from apoptotic cell death.

Regarding the Cao et al. reference, J. Neurosci. Res. 68:501-510 (2002), Applicants maintain that this reference describes the manipulation of endogenous neural precursors as challenging and unsuccessful. In contrast, the claimed methods are directed to differentiating progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide or active fragment thereof. Therefore, Applicants maintain that the issues with respect to the manipulation of endogenous neural precursors in general are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*.

Referring to Mehler et al., Arch. Neurol. 56:780-784 (1999), Applicants respectfully maintain that the issues with respect to environmental cues for differentiation of neural progenitor cells that may or may not be present in normal or neuropathological conditions are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*. Contrary to the assertion in the Office Action on page 13, Applicants respectfully maintain that the claims do recite specific steps for differentiating cells. In particular, the claims recite the steps of contacting the progenitor cells with a differentiating agent; and introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death.

Applicants respectfully submit that the issues discussed in the Office Action regarding Jackowski et al., Br. J. Neurosurg. 9:303-317 (1995), Grados-Munro et al., J. Neurosci. Res. 74:479-485 (2003), and Filbin, Nat. Rev. 4:1-11 (2003), are not relevant to the claimed methods.

In contrast, the claimed methods are directed to differentiating progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide or active fragment thereof.

Applicants respectfully maintain that Cheng et al., Blood 92:83-92 (1998), previously provided in the response filed September 3, 2004, provides corroborative evidence that one skilled in the art would have been able to introduce a nucleic acid molecule into human progenitor cells. In particular, Applicants respectfully maintain that the optimized retroviral gene-transfer protocol described by Cheng et al. is relevant to *in vivo* uses since such viral vectors are routinely used for *in vivo* transduction. Furthermore, Applicants respectfully maintain that Hanazono et al., Stem Cells 19:12-23 (2001), supports Applicants contention that viral vectors were routinely used for gene transfer into hematopoietic stem cells.

Applicants further maintain that Zwaka and Thomson, Nat. Biotechnol. 21:319-321 (2003), which was previously submitted with the response filed September 3, 2004, corroborates Applicants' position that electroporation can be used to produce stably transfected human ES cells. In the Office Action on page 14, it is asserted that the specification does not provide specific guidance for using electroporation techniques for transfecting human ES cells and that "only a laundry list" of techniques that could be used to introduce a nucleic acid molecule into an embryonic stem cell is provided. To the contrary, Applicants respectfully maintain that the specification teaches exemplary well known methods for introducing a MEF2 polypeptide into a progenitor cell such as an embryonic stem cell, including exemplary references teaching such well known techniques (see specification, for example, on page 54, lines 4-30). Applicants emphasize that the law is quite clear as to what a patent is required to teach to enable the claimed invention. The specification need not disclose, and preferably omits, that which is well-known to those skilled and already available to the public. *In re Buchner*, 929 F.2d 660, 661, 18 U.S.P.Q.2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 U.S.P.Q. 481, 489 (Fed. Cir. 1984). Applicants respectfully maintain that, based on the

teachings in the specification and what was well known to those skilled in the art, one skilled in the art would have been enabled to make and use the methods as claimed.

Regarding Eiges et al., Curr. Biol. 11:514-518 (2001), which was submitted in the response filed September 3, 2004, Applicants respectfully maintain that this reference corroborates Applicants' position that electroporation can be used to transfect human ES cells. Eiges et al. describes the successful transfection of human ES cells using electroporation and two commercially available transfection reagents, Fugene and ExGen 500, with ExGen 500 showing the highest efficiency (see Figure 1). The Office Action asserts that Eiges et al. would not have been available to the skilled artisan since it is a post-filing reference. However, Applicants respectfully maintain that Eiges et al. corroborates Applicants' position that routine transfection methods, including electroporation, could be used to successfully transfect human ES cells. Furthermore, Eiges et al. teaches that other routine transfection methods such as Fugene and ExGen 500 could also be used to successfully transfect human ES cells. As discussed in the previous response filed February 23, 2006, Applicants respectfully maintain that transfection methods such as Fugene and ExGene 500 were routine and available to one skilled in the art at the time of filing of the priority application, June 5, 2000, as corroborated by Ferrari et al., Gene Therapy 4:1100-1106 (1997), and Uyttersprot et al., Mol. Cell. Endocrin. 142:35-39 (1998). These references were provided to corroborate that the transfection methods for human ES cells described in Eiges et al. were well known and available to one skilled in the art. Applicants respectfully maintain that Eiges et al. describes the successful transfection of human ES cells using routine methods well known to those skilled in the art at the time of filing of the priority application and that it would have been routine for one skilled in the art to try various known transfection methods to successfully transfect human ES cells, as corroborated by Eiges et al.

The Office Action refers in several places to a description of "Example 6" (see, for example, the paragraph bridging pages 15-16). The description in the Office Action regarding "Example 6" appears to refer to a different specification than the instant specification. Applicants point out that Example VI of the specification describes that the inhibition of MEF2 function enhances apoptotic cell death during neuronal differentiation.

Applicants respectfully maintain, as discussed in the previous responses filed March 29, 2004, and February 26, 2006, that the claimed methods of differentiating progenitor cells do not recite, nor do they require, a particularly “high efficiency” of transfection. Rather, one skilled in the art understands that cell populations stably expressing an introduced nucleic acid molecule can be routinely prepared using, for example, standard methods such as antibiotic selection in order to select for a transfected population of cells. Thus, the skilled person understands that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able produce a population of progenitor cells predominantly or uniformly containing a MEF2 polypeptide using only routine methods.

Regarding the discussion on page 17 of the Office Action relating to Milward et al., J. Neurosci. Res. 50:862-871 (1997), Applicants discussed Milward et al. on page 16 of the previous response as corroborating that differentiation can occur *in vivo* in injury and disease. In the Office Action dated August 12, 2005, it was asserted that the references by McDonald et al., Nat. Med. 5:1410-1412 (1999), and Liu et al., Proc. Natl. Acad. Sci. USA 97:6126-6131 (2000), described “an injury model” rather than “an ongoing pathological process.” Milward et al. was discussed in the previous response as exemplifying an “ongoing pathological process” as distinct from “an injury model.” The Office Action on page 17 appears to indicate that the disease model described by Milward et al. is a genetic defect that is not considered to be “an ongoing pathological process.” Regardless of how the disease model of Milward et al. is viewed, Applicants respectfully maintain that it is clearly exemplary of disease model that is not “an injury model” and supports Applicants’ position of record.

With respect to the Rule 132 Declaration by Dr. Lipton submitted with the previous response of February 26, 2006, the Office Action indicates on page 19 that the Declaration did not provide a nexus between the experiments described in the Declaration and the teachings in the specification. To provide more details of the experiments described in the previously filed Declaration, attached herewith is another Rule 132 Declaration by Dr. Lipton (Exhibit A with Exhibits 1 through 5 attached thereto), which provides additional experimental details of the experiments described in the previous Declaration. Applicants point out that the constitutively active MEF2, referred to as MEF2CA in the Rule 132 Declaration, and the dominant negative MEF2, referred to as MEF2DN in the Rule 132 Declaration, are described in the specification,

for example, on page 71, lines 17-21, and on page 80, lines 7-18. The specification also describes using the expression of green fluorescent protein (GFP) to identify transfected cells (page 80, lines 12-18). Regarding the figures referenced in the previously filed Declaration, the Office Action indicates on page 19 that color photographs were not provided. Applicants believe that color photographs were provided with the previous response, but it is unclear whether color photographs would appear in the scanned documents of the previously submitted response as processed by the Office. To make the record clear, Applicants submit herewith color photographs of Figures 5-1 through 5-5 (Exhibits 1-5) as referenced in the Declaration submitted herewith. Applicants respectfully submit that there is an appropriate nexus between the Rule 132 Declarations previously submitted and submitted herewith and the teachings in the specification. Applicants submit that the Rule 132 Declaration describes experimental results showing that transplanted MEF2CA neural stem cells survive, migrate and differentiate into neurons in the ischemic mouse cerebral cortex. Applicants respectfully submit that the evidence provided in the attached Declaration corroborates the enablement of the claimed methods.

For the reasons of record and as discussed above, and further in view of the corroborative evidence submitted previously and herewith, Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 1-20 and 58-79 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite is respectfully traversed. Applicants respectfully submit that the claimed methods are clear and definite. The specification teaches that the invention provides a method of differentiating progenitor cells to produce a population of neuronal cells, which is protected from apoptotic cell death (page 16, line 29, to page 17, line 1). Although Applicants respectfully submit that one skilled in the art would understand the meaning of “protected neuronal cells,” nevertheless claims 1 and 59 have been amended to recite neuronal cells “protected from apoptotic cell death.” Applicants respectfully submit that the claims are clear to one skilled in the art. Accordingly, Applicant respectfully request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 102

The rejection of claims 1-4, 18, 58,62, 76 and 79 under 35 U.S.C. § 102(a) as allegedly anticipated by Okamoto et al., Proc. Natl. Acad. Sci. USA 97:7561-7566 (2000), is respectfully traversed. Applicants respectfully submit that the claimed methods are novel over Okamoto et al. Applicants point out that the priority application, serial No. 60/209,539, was filed June 5, 2000. Okamoto et al. was published online on June 13, 2000. As evidence that this reference was published on June 13, 2000, submitted herewith as Exhibit B is a printout from the journal website indicating that it was published online on June 13, 2000. Applicants further point out that the subject matter of Okamoto et al. was substantially disclosed in priority provisional application 60/209,539. Note, for example, that Figures 6-10 of the priority provisional application correspond to Figures 1-5 of Okamoto et al. Applicants respectfully submit that the priority date of the subject application is prior to the publication date of Okamoto et al. Therefore, Applicants respectfully submit that Okamoto et al. is not proper prior art. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1, 2, 18, 58-60, 76 and 79 under 35 U.S.C. § 102(b) as allegedly anticipated by Krainc et al., J. Biol. Chem. 273:26218-26224 (1998), is respectfully traversed. Applicants respectfully submit that the claimed methods are novel over Krainc et al.

In the Office Action in the paragraph bridging pages 21-22, Krainc et al. is described as disclosing that the plasmid pG/DNA, containing the N-terminal DNA binding domain of MEF2C, was stably transected into P19 cells, referring to Figure 5 and page 26222, column 2, paragraph 2. The referenced paragraph from Krainc et al. reads as follows:

To gain more direct evidence that endogenous MEF2C is involved in *NR1* gene expression *in vivo*, we monitored NR1 mRNA levels during neuronal differentiation in the presence or absence of a dominant-negative MEF2C protein. We stably transfected the plasmid pG/DN, which contains the cDNA sequence of the NH2-terminal DNA binding domain of MEF2C, into P19 cells. These cells differentiate into a neuronal phenotype after treatment with 13-*cis*-retinoic acid, and then express MEF2C (55) as well as glutamate receptor mRNAs (38). by reverse transcriptase-PCR, we observed induction of NR1 mRNA expression in P19 cells after differentiation with 13-*cis*-retinoic acid for 7 days (Fig. 5A, lane 4). This induction was totally abolished, however, in p19 cells stably expressing the dominant-negative MEF2C (Fig. 5A, lane 5). [emphasis added]

As described in the passage above and on page 26219, column 1, under “Stable Transfection,” the pGK/DN construct contains a dominant-negative MEF2C cDNA. In contrast, the claimed methods include the step of introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Krainc et al. provides no teaching of introducing into progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof. Furthermore, Krainc et al. provides no teaching that introducing a MEF2 polypeptide or active fragment thereof will produce a cell population containing neuronal cells protected from apoptotic cell death. Absent such a teaching, Applicants respectfully submit that Krainc et al. cannot anticipate the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1, 18, 20, 59, 76 and 78 under 35 U.S.C. § 102(b) as allegedly anticipated by Mao et al., J. Biol. Chem. 271:14371-14375 (1996), is respectfully traversed. Applicants respectfully submit that the claimed methods are novel over Mao et al.

In the Office Action on page 22, Mao et al. is indicated to disclose transfection of the mouse teratocarcinoma cell line P19 with a plasmid encoding MEF2A. Table 1 is referenced as describing expression of MEF2A and MASH1 during retinoic acid-induced P19 neuronal differentiation. The reference is asserted to further disclose that MEF2A and MASH1 are coordinately induced during the differentiation of P19 cells along a neuronal lineage and that, in transient transfection assays, MEF2A and MASH1 cooperatively activate gene expression.

Applicants respectfully disagree with the assertion in the Office Action that Mao et al. discloses the transfection of the mouse teratocarcinoma cell line P19 with a plasmid encoding MEF2A. To the contrary, Mao et al., at best, describes transfection experiments involving MEF2A in CV1 or COS cells, not P19 cells as asserted in the Office Action. For example, Mao et al. describes transient transfection assays in CV1 cells, in which MEF2A and MASH1 are co-transfected and shown to increase CAT reporter activity (see paragraph bridging pages 14373-14374 and Figure 2). Mao et al. also describes transfecting MEF2A and MASH1 in COS cells and performing immunoprecipitation with anti-MASH1 antibodies (page 14374, first column,

second paragraph and Figure 3). However, Mao et al. provides no teaching of transfecting P19 cells with MEF2A, as asserted in the Office Action.

Moreover, Mao et al. does not teach the claimed methods, which include the steps of contacting the progenitor cells with a differentiating agent; and introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Mao et al. provides no teaching of introducing into progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof. Furthermore, Mao et al. provides no teaching that introducing a MEF2 polypeptide or active fragment thereof will produce a cell population containing neuronal cells protected from apoptotic cell death. Absent such a teaching, Applicants respectfully submit that Mao et al. cannot anticipate the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1, 2, 18, 58-60, 76 and 79 under 35 U.S.C. § 102(b) as allegedly anticipated by Skerjanc et al., FEBS Lett. 472:53-63 (2000)(hereinafter Skerjanc et al., 2000), as evidenced by Skerjanc et al., J. Biol. Chem. 273:34904-34910 (1998)(hereinafter Skerjanc et al., 1998), is respectfully traversed. Applicants respectfully submit that the claimed methods are novel over Skerjanc et al., 2000.

Applicants respectfully submit that Skerjanc et al., 2000, does not teach the claimed methods. With respect to the comments in the Office Action on page 23 regarding this reference, first, Applicants respectfully disagree with the assertion that Skerjanc et al., 2000, “discloses that MEF2C can induce neurogenesis when overexpressed in P19 cells,” referring to page 53, column 1, paragraph 2, and abstract. As indicated in the Office Action, Skerjanc et al., 1998, describes generating the P19[MEF2C] cells, which overexpress MEF2C. As discussed in Skerjanc et al., 1998, MEF2C expression initiated cardiomyogenesis (see abstract in Skerjanc et al., 1999; see also Table 1 in Skerjanc et al., 2000), not neurogenesis as asserted in the Office Action.

Second, Skerjanc et al., 2000, indicates that “P19 cells differentiate into neuroectodermal lineages when aggregated with retinoic acid but not when aggregated with ME₂SO [DMSO] or in the absence of retinoic acid” (page 54, left column, first sentence under “Results and

discussion"). Thus, DMSO does not cause P19 cells to differentiate into neuroectodermal lineages, in contrast to retinoic acid. The specification teaches that a "differentiating agent" is a naturally occurring or synthetic cytokine, growth factor or other compound that causes or enhances a progenitor cell to have on or more characteristics of a neuronal cell (page 46, lines 18-22). Exemplary differentiating agents include retinoic acid, neurotrophic factor 3, epidermal growth factor, insulin-like growth factor-1, and platelet derived growth factor. Moreover, Skerjanc et al., 2000, does not teach the claimed methods, which include the step of introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Skerjanc et al., 2000, provides no teaching of introducing into progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof. Furthermore, Skerjanc et al., 2000, provides no teaching that introducing a MEF2 polypeptide or active fragment thereof will produce a cell population containing neuronal cells protected from apoptotic cell death. Absent such a teaching, Applicants respectfully submit that Skerjanc et al., 2000, cannot anticipate the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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